#8 C. Off. 8/16/95

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re Application of:	W. James Huang, Douglas B. Johns, Richard L. Kronenthal
Ser. No:	08/192,336
Filed:	February 4, 1994
For:	IONICALLY CROSSLINKED CARBOXYL-CONTAINING POLYSACCHARIDES FOR ADHESION PREVENTION
Examiner:	E. White
Group Art:	1803

Honorable Commissioner of Patents
and Trademarks
Washington, DC 20231

Docket No: L15.2-512

DECLARATION OF DOUGLAS B. JOHNS UNDER 37 C.F.R. §1.132

Dear Sir:

1. I, Douglas B. Johns, do hereby declare that I am a citizen of the United States and a resident of Milford, New Jersey.

2. I received a Bachelors of Science in Textile Chemistry from the Philadelphia College of Textiles and Science, Philadelphia, Pa., in 1979; a Master of Science in Polymer Science and Engineering from the University of Massachusetts, Amherst, MA, in 1981; and a Doctorate Degree in Polymer Science and Engineering from the University of Massachusetts, Amherst, MA, in 1983.

3. Since February 1984, I have been employed as a research and development scientist and manager for Ethicon, Inc. My work at Ethicon has been primarily

related to developing and evaluating absorbable polymer based medical devices (including polymers to prevent adhesions) and drug delivery systems.

4. I have read an am familiar with the contents of the above identified application.

5. I have read and am familiar with the GALATIK patent. The following comments are based on my review of that patent.

GALATIK describes his invention as relating to a pharmacological preparation for use in human and veterinary medicine. The essence of the preparation contains 0.02 to 3 wt% of an alkali metal hyaluronate complexed with a multivalent cation selected from the group consisting of Mg^{+2}, Ca^{+2}, Zn^{+2}, Ba^{+2}, Al^{+3}, Cu^{+2}, Zr^{+4}, Cr^{+3}, Fe^{+3} either alone or mixed with physiological salt solution, where the molar composition of the complex is 0.1 to 5 moles of hyaluronate and 1 to 25 moles of the coordinated cation.

Thus, GALATIK'S complex could range from 0.2 to 250 moles of cation per mole of hyaluronate:

1 mole Cation / 5 mole Hyaluronate = 0.2 mole Cat. / mole of Hy.

25 mole Cat. / 0.1 mole Hy. = 250 mole Cat. / mole of Hy.

The number of moles of the two components is based on the molecular weight of each component. For the cationic species, this is straight forward. However, for the hyaluronate, one could use the monomer molecular weight (i.e. the building block or repeat unit in the polymer chain, which for hyaluronate is a specific disaccharide, with a molecular weight of 401 g/mole for the sodium salt) or the polymer molecular weight. The polymer molecular weight varies depending on the source, but a range of "300,000 to 8,000,000 or higher" was given in GALATIK.

The examples presented in GALATIK can be examined to determine which of these, monomer or polymer, formed the basis of the calculations. Six examples are provided. Examples 2, 3, and 4 assume the reader knows the answer to the "mole question", and disclose a dimer, 1.5mer and dimer, respectively, not contemplated by the present invention. Examples 1, 5, and 6 provide sufficient information to make calculations using the different molecular weights for hyaluronate, i.e. monomer (401 g/mole) or polymer (either 300,000 or 8,000,000 g/mole). Examination of these calculations leads one to the conclusion that the polymer molecular weight had to be used in the calculations for the examples to be within the claim of 0.2 to 250 moles of cation per mole of hyaluronate.

Example 1.

Given:

0.2 mg $MgCl_2 \cdot 6H_2O \div 203.30$ mg / mmol $= 9.84 \times 10^{-4}$ mmol Cation

10.0 mg NaHy $\div$ X mg / mmol

Then,

a) where $X = 401$ mg / mmol $= 2.49 \times 10^{-2}$ mmole Hy

b) where $X = 300{,}000$ mg / mmol $= 3.33 \times 10^{-5}$ mmole Hy

c) where $X = 8{,}000{,}000$ mg / mmol $= 1.25 \times 10^{-6}$ mmole Hy

The ratio of moles of cation to moles of hyaluronate for the three values of x are:

a) 0.039

b) 29.52

c) 787.20

Thus, only "b" (300,000 g/mole) would produce a composition in the claimed range of 0.2 to 250.

Example 5.

Given:

0.001 mg $ZnSO_4 \cdot 7H_2O \div 287.54$ mg / mmol $= 3.48 \times 10^{-6}$ mmole Cation

10.0 mg NaHy $\div$ X mg / mmol

Then,

a) where X = 401 mg / mmol = 2.49×10^{-2} mmole Hy

b) where X = 300,000 mg / mmol = 3.33×10^{-5} mmole Hy

c) where X = 8,000,000 mg / mmol = 1.25×10^{-6} mmole Hy

The ratio of moles of cation to moles of hyaluronate for the three values of x are:

a) 1.39×10^{-4}

b) 0.104

c) 2.78

Thus, only "c" (8,000,000 g/mole) would produce a composition in the claimed range of 0.2 to 250.

Example 6.

Given:

0.0005 mg $Zr(SO_4)_2$ ÷ 283.34 mg / mmol = 1.76×10^{-6} mmole Cation

10.0 mg NaHy ÷ X mg / mmol

Then,

a) where X = 401 mg / mmol = 2.49×10^{-2} mmole Hy

b) where X = 300,000 mg / mmol = 3.33×10^{-5} mmole Hy

c) where X = 8,000,000 mg / mmol = 1.25×10^{-6} mmole Hy

The ratio of moles of cation to moles of hyaluronate for the three values of X are:

a) 7.08×10^{-5}

b) 5.29×10^{-2}

c) 1.41

Thus, only "c" (8,000,000 g/mole) would produce a composition in the claimed range of 0.2 to 250.

In summary, these calculations indicate that the polymer molecular weight must form the basis of the cation to hyaluronate ratio on a molar basis. Further, Example 6 had to have used a hyaluronate with a minimum molecular weight of 1,133,360 (in order to achieve the minimum ratio of 0.2).

6. Crosslinking or complexation, in my application is described in terms of the percentage of the carboxylic acid groups in the polymer which could theoretically be bound by the added cation. As stated previously, hyaluronate is composed of a disaccharide repeat unit. Each disaccharide repeat unit has one carboxylic acid group. A 100 percent crosslinked material would contain 1 trivalent ion per every three disaccharide repeat units or 1 divalent ion per every two disaccharide repeat units. Thus, a crosslinking level of 60 to 100 percent, with Fe^{+3} cation and a hyaluronate with an average molecular weight of 1,113,360 (from Example 6 above) would equate to a cation to hyaluronate mole ratio of 557 to 928.

Calculation:

Given:

10 g Hy ÷ 401 g / mole of Disaccharide = 0.025 moles of Disaccharide

60% Crosslinking with Fe^{+3}, => 0.6 x (0.025 moles ÷ 3) = 0.005 moles Fe^{+3}

and since,

10 g Hy ÷ 1,113,360 g / mole of Hy polymer = 8.98×10^{-6} moles Hy

then,

the ratio of moles of cation to moles of hyaluronate is:

$0.005 \div 8.98 \times 10^{-6} = 557$

In GALATIK, complexation involves a very small amount of cation, which on a molar ratio with the hyaluronate polymer is from 0.2 to 250 moles of cation per mole of

hyaluronate. Using the method set forth above, and in my Application, the percent crosslinking in Examples 1, 5, and 6 of GALATIK are all less than 10%, and are 7.9%, 0.03%, and 0.007% respectively. Following the same method, the maximum crosslinking density that GALATIK discloses with a trivalent cation, a molar ratio of 250 and a hyaluronate average molecular weight of 550,000 would be 55 percent. The crosslinking density for higher molecular weights of hyaluronate with a trivalent cation, and a molar ratio of 250 will all provide crosslinking percentages proportionately less than 55 percent. Furthermore, Galatik specifically teaches the use of commercially available sodium hyaluronate, HEALON, which is generally known to have a molecular weight of 1,000,000 to 2,000,000. It is therefore clear that the crosslinking density will be significantly less than 55 percent.

7. Table 6 from my Application (reproduced in part below) describes a 1% hyaluronate with no crosslinking, and 25%, 50%, and 90% crosslinking with Fe^{+3}. The corresponding viscosities are:

Hyaluronate, 1% w.	Viscosity
0% Crosslinking	1,200 cps
25%	10,200 cps
50%	39,200 cps
90%	44,900 cps

Table 4 shows a clear trend towards improved efficacy with increasing crosslinking density was observed in the rabbit sidewall studies. Ninety percent crosslinked FeHA virtually eliminated adhesions in the sidewall model. Additionally, highly crosslinked FeHA with a lower HA concentration out performed FeHA with a similar viscosity having a lower crosslinking density.

It is my opinion from reviewing the data that crosslinking, not viscosity is generating the unexpected adhesion reduction benefits. In fact, Table 4 shows that very high viscosity uncrosslinked HA is not efficacious, while all of the 90% crosslinked materials of considerably lower viscosity are efficacious.

8. I declare that all statements made herein of my own knowledge are true and all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both under §1001 of Title 18 of United States Code and that such willful false statements may jeopardize the validity of this application or any patent issuing therefrom.

Dated: 6/20/95

Douglas B. Johns

Douglas B. Johns, Ph.D.

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